

Fig. 1

Fig 1 E - cont

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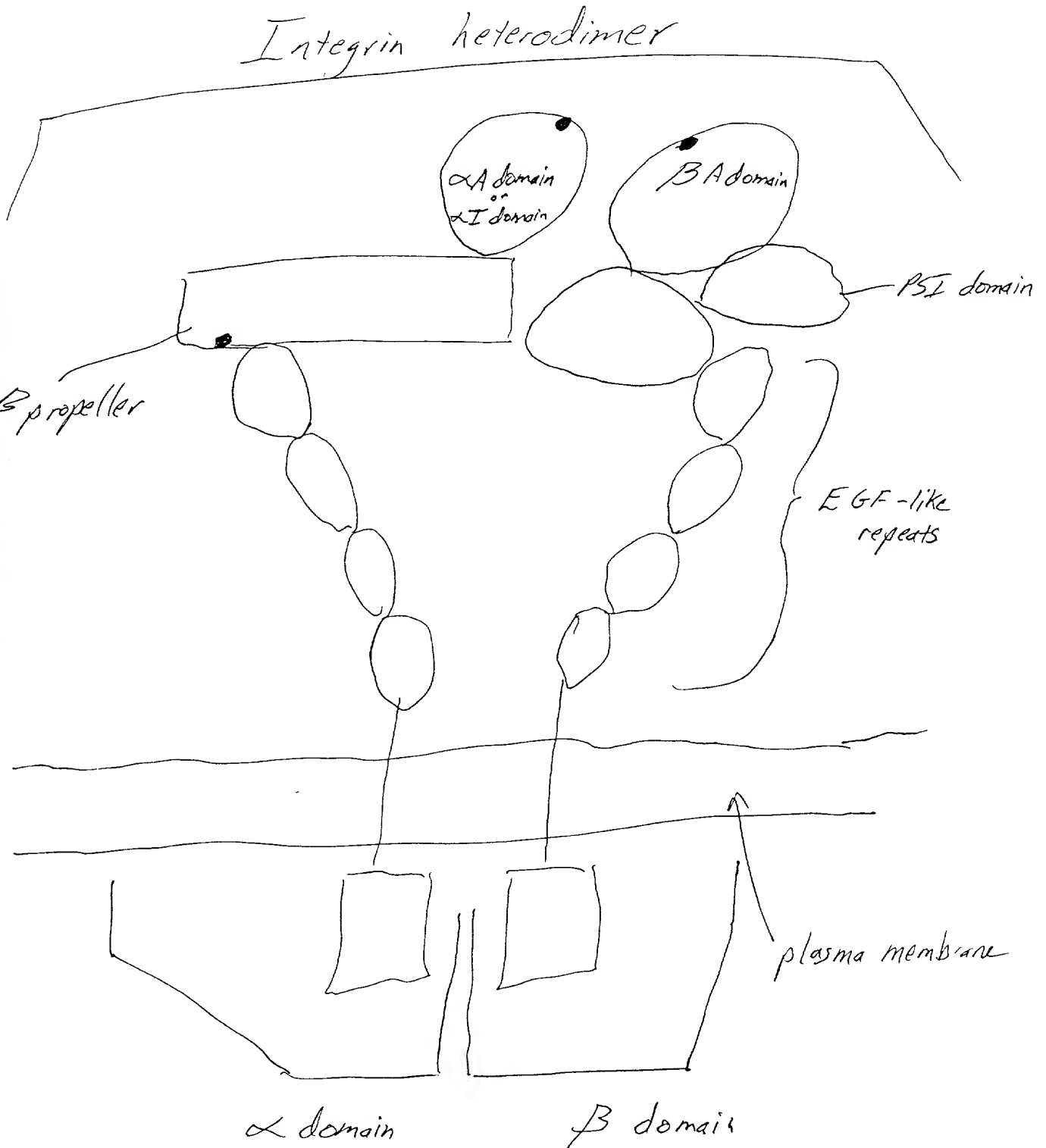


Fig 1F

05040" 1342660

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Fig 1 F

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Fig/F

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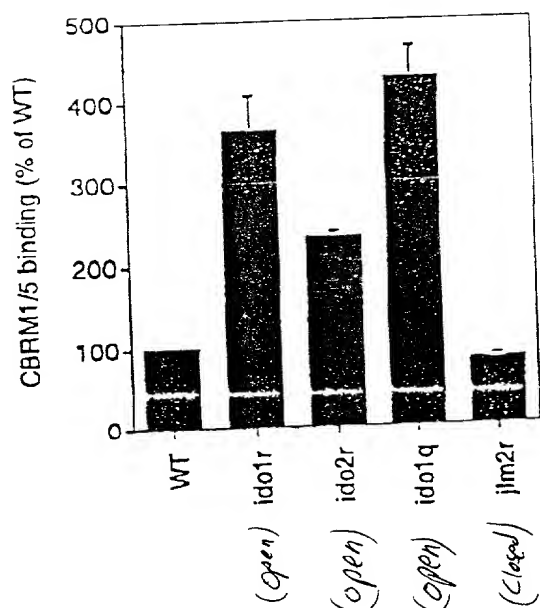
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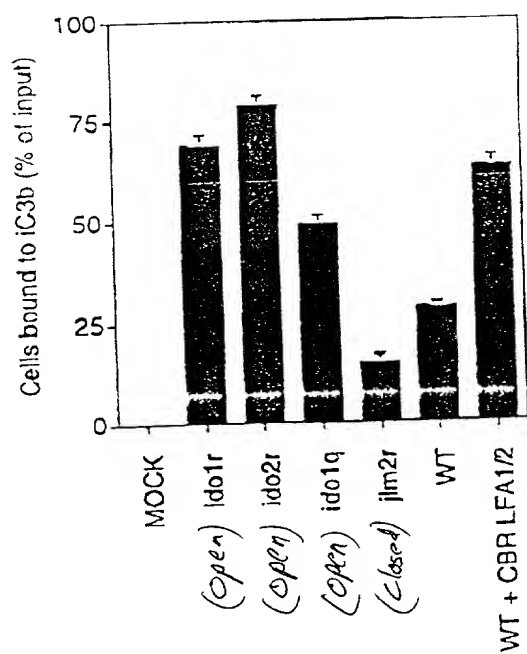
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Fig 2

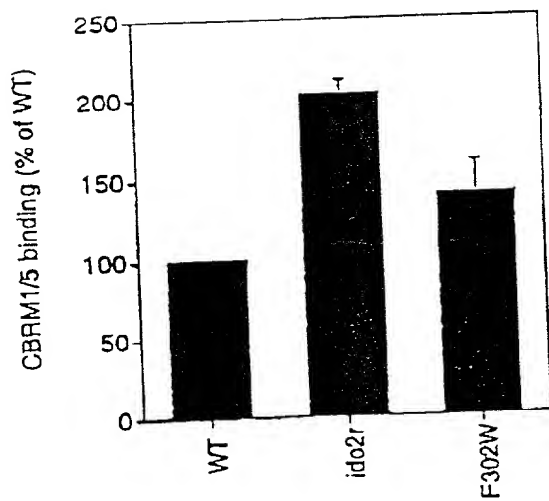
A



B



C



D

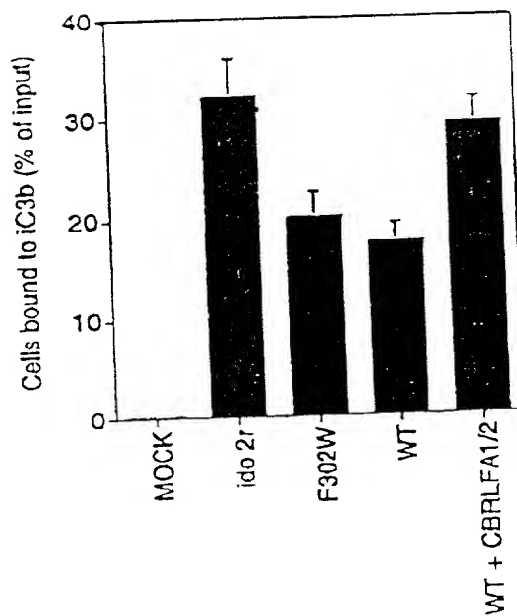


Fig. 3

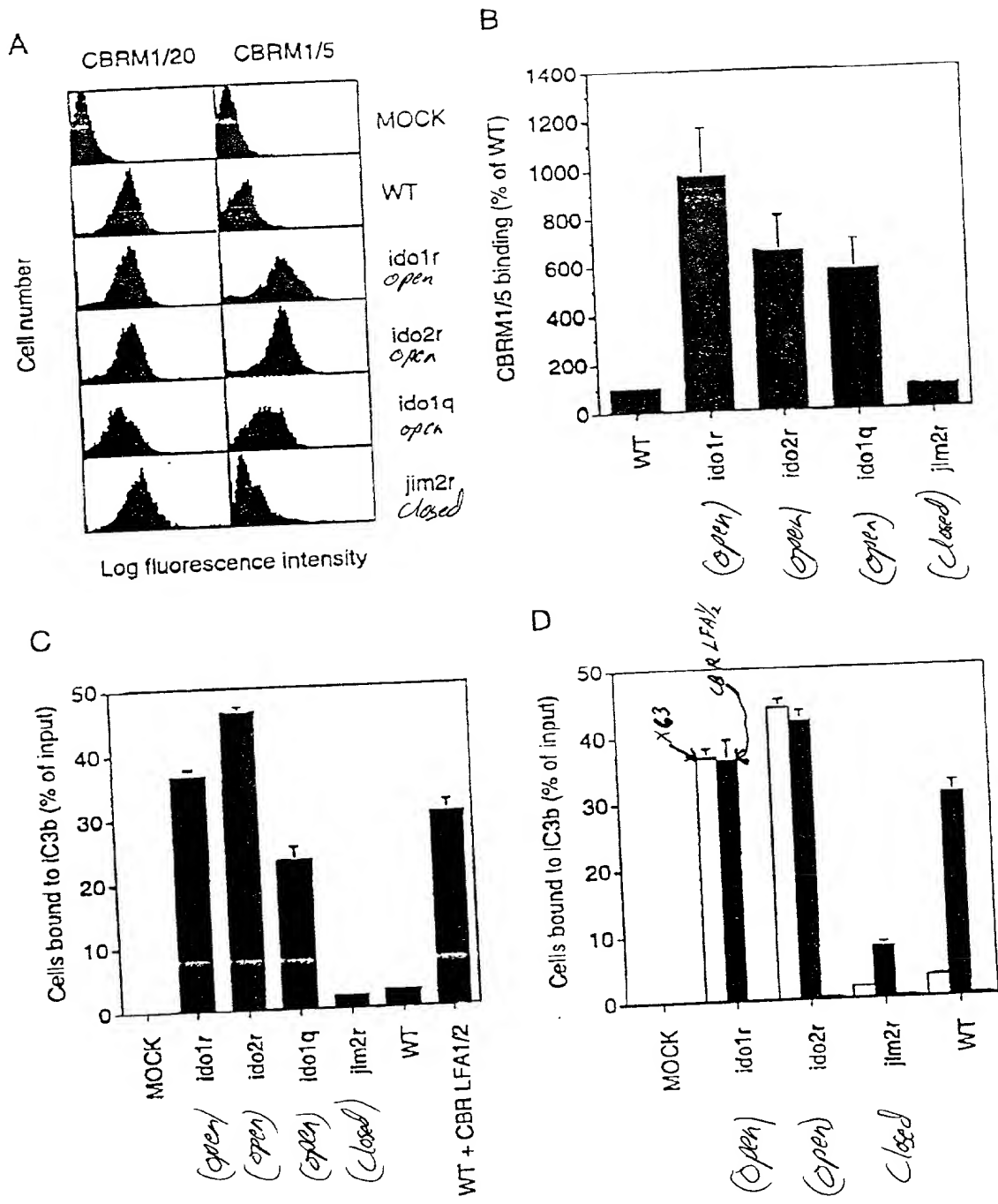
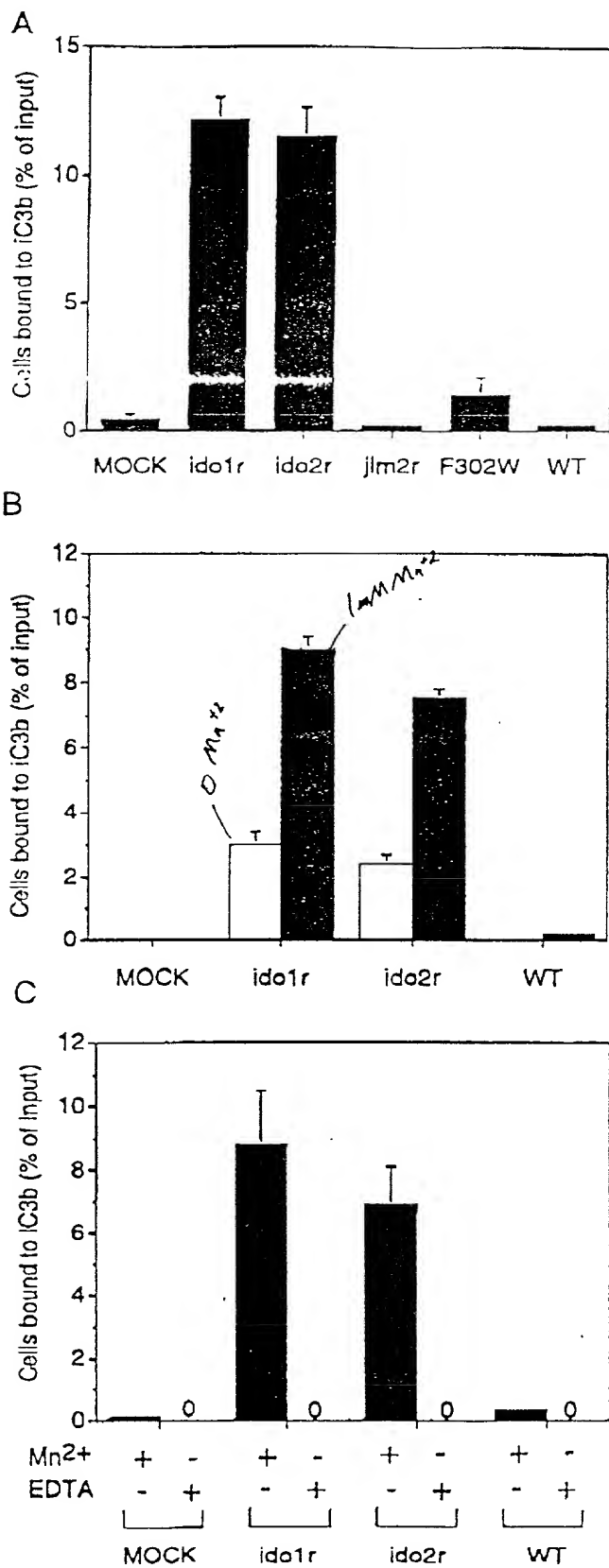


Fig 4



FO6020" F8420660

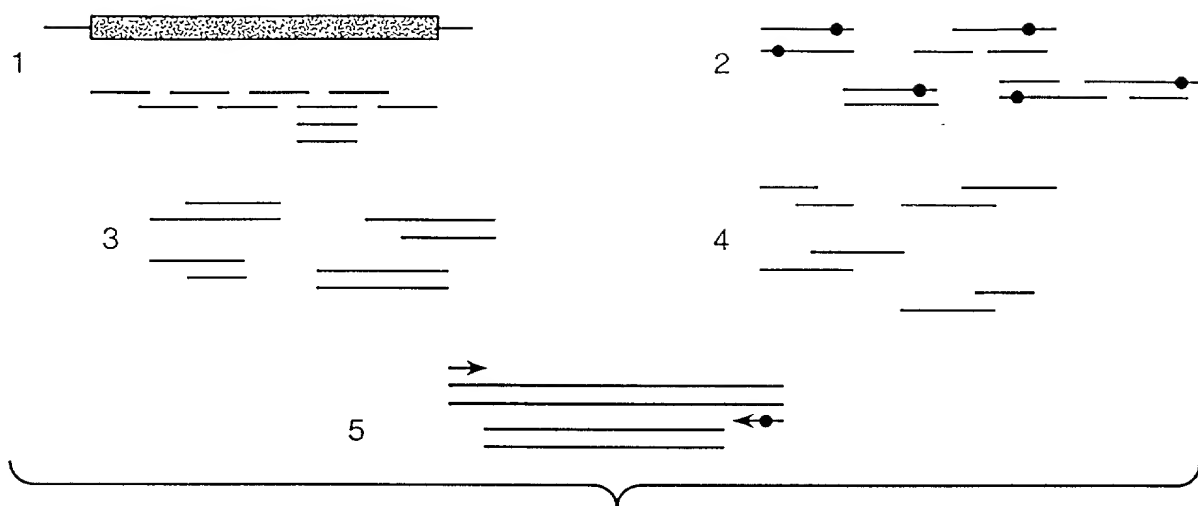


Fig 5

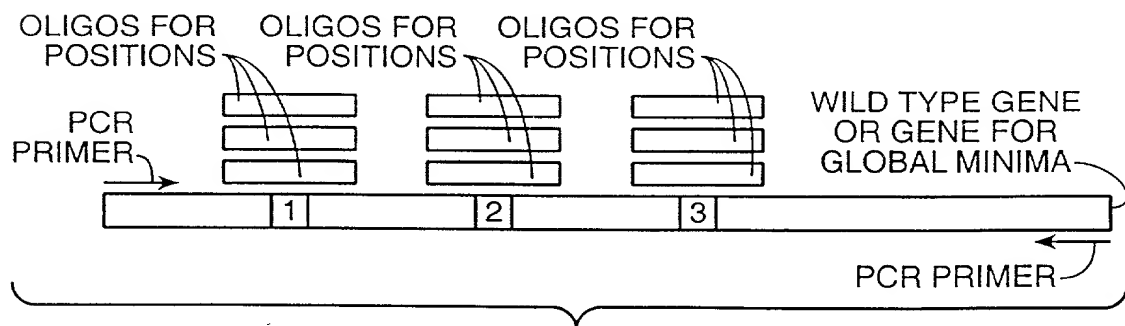
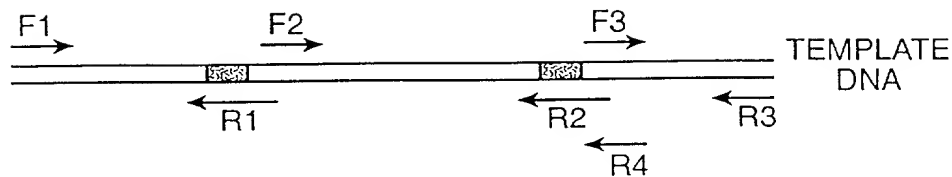


Fig 6

BLACK BOX =
REGION TO
BE MUTATED



STEP 1: SET UP 3 PCR REACTIONS:

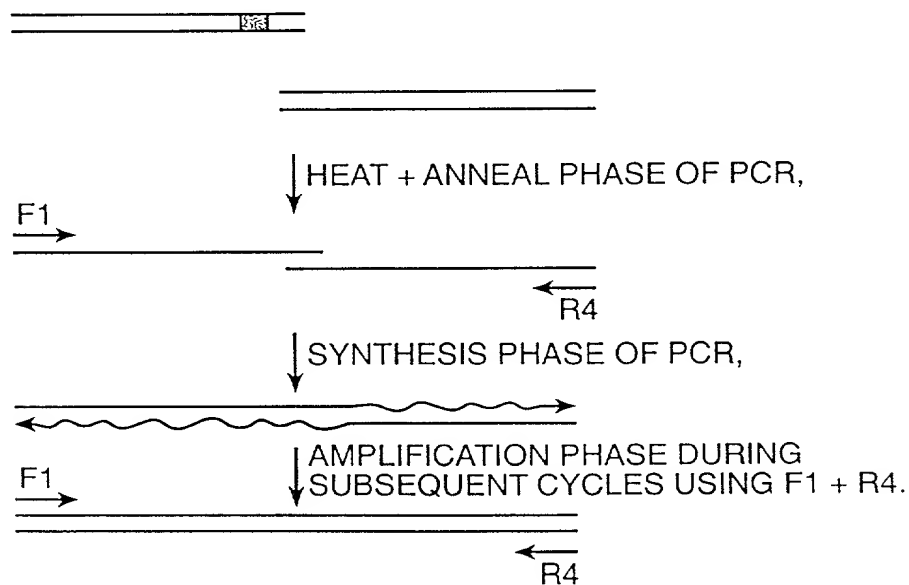
PRODUCTS:

TUBE 1:

TUBE 2:

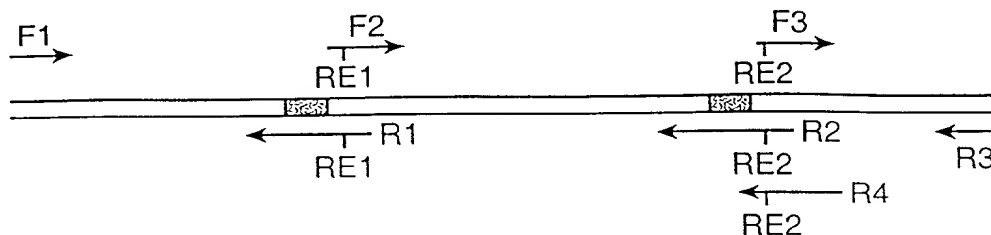
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 +
PRODUCTS TUBE 2 + F1 + R4.



STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT
FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG. 7



STEP 1: SET UP 3 PCR REACTIONS:

TUBE 1:

TUBE 2:

TUBE 3:

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



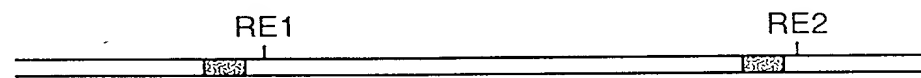
STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 3.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

Fig 8
DIAGRAM 3



Fig. 9